

Protocol for the Presumptive Color Test for GBL  
and 1,4 Butanediol Version 1.0

Prepared By John Beelle Date 9/1/04

Reviewed By Cathleen Morrison Date 9-1-04

Reviewed By Carl W. Schlander Date 9/2/04

## Introduction:

This is a presumptive color test that may be used to screen a sample for the presence of Gamma-butyrolactone (GBL) and 1,4 Butanediol (1,4 BD). It is also a good presumptive color test to rule out the presence of Gamma-hydroxybutyrate (GHB), since no color change takes place in compounds containing only GHB. GHB is the target analyte in most investigations, but GHB precursors (GBL and 1,4 BD) are becoming more and more popular. They both metabolize into GHB when ingested.

With this method, the color change for GBL and 1,4 BD is blue. GBL gives a more intense blue, and 1,4 BD gives a faint blue color. Further confirmatory analysis (FTIR, GC/MS) can confirm the presence of GBL or 1,4 BD.

The initial color of the sample solution should have no effect on the resulting blue color, since the GBL and 1,4 BD are extracted into a toluene layer which is clear, prior to being added to Cobalt Thiocyanate crystals in a spot well.

## Safety:

All safety requirements as outlined in the Drug Unit and Lab-wide safety manuals should be followed. Note that some of the materials may be toxic, and familiarity with MSDS's is recommended.

## Materials Needed:

Gamma-hydroxybutyric Acid (GHB)  
Gamma-butyrolactone (GBL)  
1,4 Butanediol (1,4 BD)  
Cobalt Thiocyanate  
Toluene  
Distilled Water

## GBL and 1,4 Butanediol Spot Test:

1. Add approximately 1-2ml of straight sample solution into a test tube.
2. Add approximately 1-2ml of toluene to the test tube containing the sample solution and cap.
3. Vortex and let stand. You should have two layers.
4. Place a few crystals of Cobalt Thiocyanate into a spot well.
5. Add 3-5 drops of the toluene layer (Top Layer) to the Cobalt Thiocyanate.

A blue color will develop in the liquid portion (toluene) after being placed onto the cobalt thiocyanate crystals if GBL and/or 1,4 BD is present. **GHB does not** produce a color change in this spot test. The toluene will remain clear after being placed onto the cobalt thiocyanate crystals if GHB alone is present.

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## Protocol for the Presumptive Color Tests for GBL and 1,4 Butanediol

The negative control consists of distilled water being mixed with toluene (in the same manner as the sample liquids). A few drops of the toluene layer are added to the cobalt thiocyanate crystals. The toluene should remain clear. A positive control consists of GBL or 1,4 BD standard in water being mixed in a 1:1 volume : volume ratio with toluene (in the same manner as the sample liquids). A few drops of the toluene layer are added to the cobalt thiocyanate crystals. The toluene should turn blue.

Note: False positives may be observed with methanol, ethyl alcohol, and chloroform. These alone produce a blue color when added to cobalt thiocyanate. Therefore, if any starting sample contains these chemicals, a false positive can be indicated. A false positive consists of the cobalt crystals turning blue, but the toluene turning a light purple color. A true positive consists of the cobalt crystals slightly turning blue and the toluene turning blue or faint blue. The more concentrated sample containing GBL or 1,4 BD, the more intense the blue color is.

#### Quality Control Procedure of Reagents:

All of the guidelines outlined in the Drug Unit Quality Control Manual will apply to the reagents, with this additional information. Each time, a negative and positive control (GBL or 1,4 BD) must be run, to insure proper reagent conditions. The reagents should be stored in their proper location when not in use.

#### References:

1. Microgram, Volume XXXII, No. 8, August 1999